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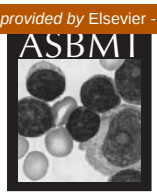

Analysis of Risk Factors for the Development of GVHD After T Cell–Depleted Allogeneic BMT: Effect of HLA Disparity, ABO Incompatibility, and Method of T-Cell Depletion

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ABSTRACT

Multivariate analysis was performed to determine the independent factors affecting the risk of acute GVHD (aGVHD) grades II to IV and extensive chronic GVHD (cGVHD) and the rate of survival in 481 recipients of T cell–depleted (TCD) marrow allografts who received transplants at a single center between 1991 and 2000. All patients received grafts partially depleted of $CD3^+$ T cells by complement-mediated lysis using 2 narrow-specificity monoclonal antibodies (MoAbs), T10B9.1A-31 (n = 400) or Muromonab-Orthoclone OKT3 (n = 81). Factors considered in the analysis included patient/donor sex, age, cytomegalovirus (CMV) status, and ABO blood group along with T-cell dose, disease and disease status, donor relationship, HLA antigen (Ag) mismatch (MM), growth-factor use, anti-thymocyte globulin use, year of transplantation, and the MoAb used for TCD. The results showed an association of HLA MM with an increased relative risk (RR) of aGVHD for recipients of grafts from related donors that were ≥2 Ag MM (n = 73, RR = 2.09, *P* = .005), matched unrelated (UR) donors (n = 130, RR = 1.98, *P* = .004), and ≥2 Ag MM UR donors (n = 34, RR = 2.68, *P* = .003) compared with the baseline matched-sibling group (n = 121). No increased risk of aGVHD was seen for 0 to 1 Ag MM family donors (n = 24) or 1 Ag MM UR donors (n = 99). aGVHD risk was increased with minor, but not major or major-minor, ABO disparity (RR = 2.0, *P* = .003) compared with that of ABO-identical pairs. We found less effective TCD and resultant higher T-cell dose for recipients of grafts that were T cell depleted using OKT3. However, the use of OKT3 and not the T-cell dose was associated with increased aGVHD risk (RR of 1.84, *P* = .001). Increased risk of extensive cGVHD was associated with patient age of >20 years (RR = 2.2, *P* < .0001) and with CMV status (positive patient/negative donor, RR = 1.9, *P* = .002). Decreased survival was associated with older age (>20 years), a ≥2 Ag MM related donor, a 1 or ≥2 Ag MM UR donor, risk group, and a CMV-positive patient/-negative donor pair. There was no difference in survival for 0 to 1 Ag MM related or matched UR donors compared with the baseline group. These data indicate that there are quantitative as well as potential qualitative differences in outcome depending on the TCD method. Expected and unexpected risk factors for GVHD and survival were associated with partial TCD. Our data support the consideration of ABO match in donor selection, the preferential selection of CMV-positive donors for CMV-positive recipients, and the acceptance of 1 but not ≥2 Ag HLA MM donors.

KEY WORDS

Bone marrow transplantation • T-cell depletion • Graft-versus-host disease • ABO compatibility • CMV serostatus

INTRODUCTION

Graft-versus-host disease (GVHD) is one of the more serious morbidities associated with bone marrow transplantation (BMT) and as such serves as a major barrier to transplantation from unrelated or partially matched family member donors [1-3]. T-cell depletion (TCD) of marrow allografts by almost every method described reduces the incidence and severity of acute GVHD (aGVHD), and, if depletion is extensive, the incidence of chronic GVHD (cGVHD) may be reduced as well [4,5]. However, despite the reduction in GVHD, TCD has not resulted in an overall increase in survival in most reported studies [3,4]. The failure to improve survival rates may, in part, result from one or all of the risks associated with TCD, namely a higher rate of graft failure, an increase in relapse for some diseases, and a delay in immune reconstitution compared with conventional allografts [4,6]. The likelihood of these risks is not the same for all forms of TCD [4]. Graft rejection and leukemia relapse appear to be more likely when TCD is rigorous, supporting the premise that both effects may be due to loss of alloreactive T cells in the graft that could eliminate residual host-derived T cells or due to leukemia that survives the conditioning regimen [7]. The effect of TCD on immune reconstitution compared with conventional allografts also varies depending primarily on the degree of TCD [8]. Relatively modest delays may be seen for TCD protocols that do not require additional immune suppression for GVHD prophylaxis [8-10]. However, profound delays in immune reconstitution may be seen in the allogeneic setting when TCD is nearly complete [11,12]. The combination of less rigorous TCD and additional immune suppression to prevent GVHD or promote engraftment can also result in a longer period of immune deficiency compared to that seen in recipients of conventional marrow allografts [10,13-15].

A recent International Bone Marrow Transplant Registry (IBMTR) study has compared the outcome of unrelated donor transplantation performed using different forms of TCD with that performed using non-TCD transplantation [4]. TCD methods were categorized into 2 main groups, those narrowly targeting T cells and those that removed a broader spectrum of cell types, including T cells. It was found that all methods of TCD reduced aGVHD risk and did not result in a higher rate of relapse compared with conventional transplantation performed using cyclosporine and methotrexate. No significant differences in outcomes were seen between TCD methods within the 2 main groups, but patients receiving grafts that were T cell depleted using narrow-specificity techniques had lower treatment failure risks compared with patients receiving transplants that were T cell depleted using broad techniques. It was speculated that there might be differences between methods of TCD within these 2 main categories that could not be ascertained due to the limitations imposed by the information available for the analysis. Our method of TCD using the narrow-specificity antibody $T_{10}B_9$-1A.31 ($T_{10}B_9$) has resulted in a reduction in GVHD incidence and severity compared with conventional allografts, without significant graft rejection, even in a setting of alternative donor transplantation [16-20]. Recently, we have modified our method of TCD to use a second narrow-specificity antibody, Muromonab-Orthoclone OKT3 (OKT3) (Ortho Biotech, Raritan, NJ), providing us the opportunity to compare outcomes using these 2 approaches at a single center as well as to determine other significant factors that affect outcome in our patient group. To this end, we performed a retrospective study of our patients treated over the past 9 years with a standardized conditioning regimen and GVHD prophylaxis schedule that includes a partially TCD BMT. We compared the TCD efficiency of the 2 monoclonal antibodies (MoAbs) and performed a multivariate analysis to determine factors associated with aGVHD (grades II-IV), extensive cGVHD, and survival.

PATIENTS AND METHODS

Patients

Between June 1991 and June 2000, a total of 503 patients received primary transplants of TCD marrow for malignant and nonmalignant diseases. All transplantation was performed at the Medical College of Wisconsin at Froedtert Memorial Lutheran Hospital, John L. Doyne Hospital, or the Children's Hospital of Wisconsin. Informed consent was obtained from each patient (or patient's guardian), and all treatment was administered under approved protocols of the Institutional Review Committee. Because T-cell content of the graft was a primary analysis factor, only the 481 patients for whom evaluable data for T-cell dose was obtained from limiting dilution assay (LDA) were included in the analysis. Six patients died prior to transplantation; thus, LDA was not performed, and 15 patients were excluded because 1 or more assay attempts failed for technical reasons. One additional patient was excluded who received a transplant from an identical twin, and 2 patients were included who received transplants in early 1990 and had retrospective LDA data. All patients received marrow that was T cell depleted by complement-mediated lysis using either $T_{10}B_9$ (n = 400) or OKT3 (n = 81) MoAb to remove $CD3^+$ T lymphocytes.

Patients received a standard conditioning regimen of intravenous cytarabine (3 g/m^2 every 12 hours for 6 doses on days –7 to –4), cyclophosphamide (45 mg/kg given 6 hours after the second and fourth doses of cytarabine), methylprednisolone (a total of 2 doses, 1 gm/m^2 at 12-hour intervals on days –2 and –1), and 13.3 or 14 Gy total body irradiation [21]. The cytarabine dose was reduced 25% to 50% for those patients older than 40 years (n = 76) at the discretion of the attending physician. Patients with aplastic anemia were treated in an identical fashion, with the exception that 16 of the 32 patients were given anti-thymocyte globulin (ATG) (Upjohn, Pharmacia, Peapack, NJ) at a dose of 15 mg/kg per day on days 4 through 10 (total 7 doses) as a result of a protocol modification made in August 1994 to promote engraftment [22].

All patients received GVHD chemoprophylaxis consisting of cyclosporine administered as an intravenous infusion beginning day –1 at 3 mg/kg per day and eventually changed to a corresponding oral dose when tolerated. Recipients of allografts from haplotype identical donors (n = 54) received ATG, 15 mg/kg per day for either 7 or 14 days beginning on day +4. Methylprednisolone was given in 2 daily doses of 1 mg/kg per day on days +2 through +17 and 1 dose on day +18 and then tapered through day +35. An additional 11 recipients of unrelated marrow received an identical course of ATG due either to presence of a 1-antigen (Ag)

Table 1. *Patient, Donor, and Graft Characteristics**

Age at transplantation, y, median (range)	
Patient	**17 (<1-62)**
Donor	**36 (<1-68)**
Year of transplantation	
1991†-1993	**144 (30%)**
1994-1996	**174 (36%)**
1997-2000	**163 (34%)**
Sex match, patient/donor	
Female/male	**156 (32%)**
Male/male‡	**123 (26%)**
Male/female	**106 (22%)**
Female/female	**96 (20%)**
Diagnosis at transplantation	
Acute leukemia‡	**248 (51%)**
Chronic leukemia	**91 (19%)**
Lymphoma	**46 (10%)**
Aplastic anemia	**32 (7%)**
Other	**34 (7%)**
Diagnosis status risk at transplantation§	
Standard‡	**140 (29%)**
Intermediate	**191 (40%)**
High	**116 (24%)**
Other	**34 (7%)**
HLA matching and donor relationship	
HLA identical siblings‡	**121 (25%)**
0-1 Ag MM related	**24 (5%)**
≥2 Ag MM related	**73 (15%)**
Matched unrelated	**130 (27%)**
1 Ag MM unrelated	**99 (21%)**
≥2 Ag MM unrelated	**34 (7%)**
ABO Matching	
Matched‡	**266 (55%)**
Minor MM	**96 (20%)**
Major MM	**90 (19%)**
Major-minor MM	**29 (6%)**
CMV serology status, patient/donor	
Positive/positive	**86 (18%)**
Positive/negative	**98 (20%)**
Negative/positive	**86 (18%)**
Negative/negative‡	**220 (46%)**
Anti-thymocyte globulin‖	
Yes	**81 (17%)**
No‡	**400 (83%)**
Growth factor†	
None‡	**258 (54%)**
G-CSF	**104 (22%)**
GM-CSF	**64 (13%)**
G-CSF + GM-CSF	**55 (11%)**
Antibody used for TCD	
$T_{10}B_9$‡	**400 (83%)**
OKT3	**81 (17%)**
T-cell dose, median (range)	**3.6×10^5/kg (4.4×10^3-12.0×10^6)**

*Except where noted, the data represent the number of patients in each group and the percentage of the total patients in that group.

†Two patients received transplants in 1990.

‡Reference group used for multivariate analysis.

§Standard risk: CR1, CP1 and SAA; intermediate risk: CR2+, PR1, Rel1, AP1, and MDS refractory anemia; high risk: relapse or blast phase or never in remission.

‖Considered only if initiated during the first week posttransplantation.

HLA mismatch (MM) or because TCD achieved less than a 1.0 log reduction, as measured by flow cytometric analysis. No patients received ATG before transplantation.

Engraftment was defined as the first of 3 consecutive days in which the absolute neutrophil count (ANC) was ≥500/mm^3. Trilineage engraftment was documented by bone marrow examination in the majority of patients 3 to 4 weeks after transplantation. Follow-up marrow studies were done at 100 days, 6 months, 1 year, and at least yearly after transplantation, whenever possible, to evaluate engraftment and disease status. Durable engraftment was confirmed by cytogenetic analysis, restriction fragment length polymorphism (RFLP) studies, or analysis of variable number of tandem repeats (VNTRs) in blood or marrow samples to distinguish donor from recipient cells. Patient, donor, and graft characteristics that were considered in the analysis are listed in Table 1.

Factors Assessed

Outcomes. The depleting efficiencies of the 2 antibodies measured as log depletion were compared. aGVHD grades II to IV, extensive cGVHD, and overall survival rates were also assessed. aGVHD was graded as 0 to IV according to criteria of Glucksberg and colleagues, whereas cGVHD was defined as none, limited, or extensive [23,24]. Patients who had evidence of engraftment were evaluable for aGVHD (n = 446). Nonevaluable patients included 15 patients who died of infection or conditioning toxicity prior to engraftment and 20 patients (4.3%) with primary graft failure, likely due to immunologic rejection. Patients who engrafted and also survived more than 90 days were evaluable for cGVHD (n = 361). The median follow-up time of survivors was 51 months (range, 4-124 months).

Variables. The variables that were assessed in this analysis for all outcomes are included in Table 1. The analysis included 2 strata based on the median of the sample with respect to the age of the patient and the donor: aged ≤20 years versus >20 years and ≤40 years versus >40 years, respectively. Risk strata were defined based on disease stage for leukemia patients as standard (complete response [CR1] or first chronic phase [CP1]), intermediate (first partial response [PR1], first relapse [Rel1], second or higher complete response [CR2+], CP2+, first accelerated phase chronic myeloid leukemia [CML] [AP1]), or high (Rel2+, AP2, blast-phase CML, or never in remission). Lymphoma patients were stratified as intermediate (CR2+) or high (relapsed or refractory). Myelodysplastic syndrome (MDS) patients with only refractory anemia were classified as intermediate risk; the remainder were considered high risk because of their overall inferior survival probability with allogeneic transplantation compared with standard- or intermediate-risk leukemia patients. Aplastic anemia (n = 32) patients were considered as standard risk, patients with immune deficiencies (n = 12) and the remaining other patients (n = 22) were grouped together because there was no standard way to subgroup them for transplantation outcome. Patients with acute lymphoblastic leukemia (ALL) in CR1 were offered transplantation only in the presence of other poor prognostic factors, such as the presence of Ph1 or other chromosomal abnormalities or age of <1 year. In the majority of patients with acute nonlymphoblastic

leukemia (ANLL) in CR1 (18 of 28), the ANLL was secondary to MDS and thus considered an indication of poor prognosis, and some patients had other poor prognostic features. Early in the program, patients with good-prognosis ANLL were offered transplantation if HLA-identical siblings donors were available. Year of transplantation was stratified into 3 roughly equal groups: 1991 to 1993, 1993 to 1996, and 1997 to 2000.

HLA matching considered 8 alleles and was based on HLA-A, HLA-B (class I) and HLA-DRB1, HLA-DQB (class II). All patients were prospectively typed for class II at the DNA level either using oligotyping [25] or DNA sequencing [26]. All patients were typed for class I by serology. A subset of partially matched family and unrelated donor/recipient pairs were typed retrospectively (n = 130) by full-length class I DNA sequencing [27] and/or 1-dimensional isoelectric focusing [28] or prospectively (n = 65) by exon 2 and 3 DNA sequencing. Antigen match was assigned based on the highest level of typing performed considering only the 8 alleles listed above.

ABO blood groups were considered matched if donor and recipient were ABO identical. A minor ABO-mismatched pair was defined when the donor uniquely possessed antibodies specific for patient ABO antigens. A major ABO-mismatched pair was defined when the patient uniquely possessed antibodies specific for donor ABO antigens. Major-minor ABO mismatch was defined for donor/recipient pairs that reciprocally possessed specific ABO antibody, eg, type A donor to type B recipient or vice versa.

ATG use was considered in the analysis only if started during the first week posttransplantation. Patients typically received ATG starting on day +4 for 7 or 14 days for reasons described above.

Patients were stratified based on the type of growth factor used posttransplantation to facilitate engraftment. Patients who did not receive growth factor or who started growth factor after the first week posttransplantation were in the no growth factor group. Granulocyte-colony–stimulating factor (G-CSF) was administered at 5 μg/kg subcutaneously (SC) per day (or the total daily dose was rounded to the nearest vial size, usually 480 μg). Granulocyte-macrophage (GM)-CSF was administered at 250 to 500 $\mu g/m^2$ SC per day. A subset of 55 patients received both G-CSF and GM-CSF during week 1 post-BMT as part of a clinical protocol. When used, growth factors were administered until sustained neutrophil engraftment was 1.0×10^9/L. The decision to use growth factors for a given patient group and the growth factor used was a function of the BMT program policy in place at the time of transplantation.

Prior to 1998, marrow grafts were depleted of T cells using the αβ T-cell–receptor antibody, $T_{10}B_9$, and baby rabbit complement [29]. Due to the unavailability of an in vivo grade of $T_{10}B_9$, TCD using OKT3 MoAb plus complement was phased in beginning in July 1998 for recipients of haploidentical marrow grafts and in October 1998 for all other recipients. For both forms of TCD, the T-cell content was measured both by flow cytometry and by a sensitive LDA for total clonable T cells [19]. The data were analyzed as T cells/kg infused. The association of T-cell content and outcome was assessed as both above and below the median and per log of T cells infused.

Statistical Analysis

The univariate (unadjusted) probability of overall survival was calculated using the Kaplan-Meier product-limit estimator. Cumulative incidences were estimated for aGVHD and cGVHD. A Student *t* test was used to compare the mean log depletion achieved with the 2 MoAbs and a Mann Whitney to compare the postdepletion T-cell dose with the 2 different antibodies. Association between the factors shown in Table 1 and the outcomes of interest were evaluated in multivariate analyses using Cox proportional hazards regression. Continuous variables were discretized in the model based on the maximum partial likelihood ratio in the Cox model. Data from patients who did not reach a given endpoint were censored at the time of death, second BMT or immunotherapy, or last assessment. Only those factors significantly ($P < .05$) associated with an outcome were retained in the final models.

For each variable and outcome, the assumption of proportional hazards was tested using a time-dependent covariate. When this result indicated differential effects over time (nonproportional hazards), models were constructed breaking the posttransplantation course into 2 time periods, using the maximized partial likelihood method to find the most appropriate breakpoint. Forward stepwise variable selection at a 0.05 significance level was used to identify covariates associated with the outcome. First-order interactions between all significant covariates were considered. Overall covariate effects were tested using the Wald test. All *P* values are 2-sided.

RESULTS

Acute GVHD

The cumulative incidence of aGVHD by day 100 posttransplantation was 33% (95% confidence interval [CI], 29%-38%) for grades II to IV aGVHD and 11% (95% CI, 9%-14%) for the severe form of the disease (≥grade III). Independent risk factors for grades II to IV aGVHD that were identified by the multivariate analysis included ABO matching, the antibody used for TCD, and HLA matching (Table 2).

Patients who had a minor ABO mismatch with the bone marrow donor had a relative risk (RR) for aGVHD grades II to IV of 2.0 ($P < .001$) compared with an ABO-identical donor/recipient pair. However, neither a major ABO mismatch nor a major-minor mismatch was associated with a greater risk of aGVHD. This increased risk of aGVHD for recipients of minor ABO-incompatible grafts was also seen for patients with severe (≥grade III) aGVHD, as shown in Figure 1. The 100-day cumulative incidence of grades II to IV aGVHD was 28% (95% CI, 13%-44%) for the baseline ABO-matched group, 48% (95% CI, 38%-58%) for the minor mismatched group, 34% (95% CI, 24%-44%) for the major mismatched group, and 28% (95% CI, 13%-44%) for the major-minor mismatch group ($P = .02$). For severe aGVHD, the cumulative incidences were 9% (95% CI, 6%-13%) for the baseline matched group, 20% (95% CI, 14%-28%) for the minor mismatched group, 11% (95% CI, 6%-18%) for the major mismatched group, and 7% (95% CI, 1%-19%) for the major-minor mismatched group ($P = .05$), as shown in Figure 1.

Table 2. *Multivariate Analysis for Acute GVHD*

Variable	RR (95% CI)	P
ABO match		
Matched	1.0	.002*
Minor mismatch	2.00 (1.37-2.92)	<.001
Major mismatch	1.13 (0.73-1.74)	.59
Major-minor mismatch	0.83 (0.39-1.74)	.62
Antibody used for TCD		
$T_{10}B_9$	1.0	
OKT3	1.84 (1.27-2.67)	.001
HLA mismatch		
Genotypically matched related	1.0	.01†
0-1 Ag related	1.22 (0.50-2.97)	.20
≥2 Ag related	2.09 (1.25-3.51)	.005
Unrelated matched	1.98 (1.24-3.15)	.004
1 Ag unrelated	1.28 (0.75-2.18)	.37
≥2 Ag unrelated	2.68 (1.41-5.13)	.003

*Three *df* test.
†Five *df* test.

Beginning in 1998, due to the unavailability of an in vivo–grade source of $T_{10}B_9$, our program switched to OKT3 for our complement-mediated lysis method of TCD. Comparison of grafts that were T cell depleted using $T_{10}B_9$ versus OKT3 revealed a lower log depletion of total clonable T cells for the OKT3-treated grafts. Mean log depletion was 1.94 (95% CI, 1.48-2.4) for $T_{10}B_9$ versus 1.69 (95% CI, 1.29-2.09), $P < .01$ for OKT3, resulting in a somewhat larger T-cell dose (median 5.4×10^5/kg for OKT3 versus 3.4×10^5/kg for $T_{10}B_9$, $P = .0005$) for recipients of grafts treated with OKT3. Because of concern over this higher T-cell dose, we performed a univariate analysis of aGVHD risk based on the antibody used for TCD. This analysis indicated a significantly higher incidence of overall aGVHD, 48% (95% CI, 37%-58%) in the OKT3 group compared to 30% (95% CI, 26%-35%, $P = .002$) in the $T_{10}B_9$ group. Similarly, the incidence of severe aGVHD was higher in the OKT3 group, 22% (95% CI, 14%-32%) compared to 9% (95% CI, 7%-12%) in the $T_{10}B_9$ group ($P = .0005$). This finding was maintained after adjustment for other significant variables (including T-cell dose) in the multivariate analysis, with an RR of grades II to IV aGVHD for the OKT3 group that was 1.84 compared to the $T_{10}B_9$ group, $P = .001$.

Recipients of HLA-identical sibling transplants served as the baseline group, with a cumulative incidence of grades II to IV aGVHD of 18% (95% CI, 11%-26%) and of severe aGVHD of 4% (95% CI, 2%-9%). There was no increased risk of overall aGVHD compared with that in genetically identical sibling transplantation for the group of patients who received marrow from related donors who were matched for 1 haplotype and either phenotypically identical or mismatched for only 1 known HLA allele on the second haplotype. However, recipients of related donor grafts who were mismatched for 2 or more HLA alleles were at higher risk for aGVHD (RR = 2.1, $P = .005$). Recipients of phenotypically HLA-identical UR donor grafts were also at increased risk for aGVHD (RR = 1.98, $P = .004$). The highest RR of aGVHD was seen for recipients of UR donor marrow with 2 or more identified HLA disparities (RR = 2.68, $P = .003$). Surprisingly, the risk of aGVHD for recipients of 1 Ag MM UR grafts was not significantly different from HLA-matched siblings in the multivariate analysis. These results are shown graphically in Figure 3.

Chronic GVHD

Extensive cGVHD was evaluable in the 368 patients who were alive and did not receive a second transplant, donor leukocyte infusion, or other cellular immunotherapy before day 90. Both patient age and cytomegalovirus (CMV) status were identified as significant independent variables in the multivariate analysis (Table 3). Patients aged >20 years were more likely to have extensive cGVHD (RR = 2.23, $P < .0001$). CMV-seropositive patients who received transplants from CMV-seronegative donors were the only group at higher risk for extensive cGVHD compared with the baseline CMV negative/negative group (RR = 1.86, $P = .002$).

Survival

The 3-year probability of survival for the group was 44% (95% CI, 40%-49%). The independent significant risk factors for survival by multivariate analysis were recipient age, HLA-matching, risk, and recipient/donor CMV serostatus at transplantation. These results are shown in Table 4. The RR of death for patients aged >20 years was 1.63 compared to the baseline younger patients, $P < .001$.

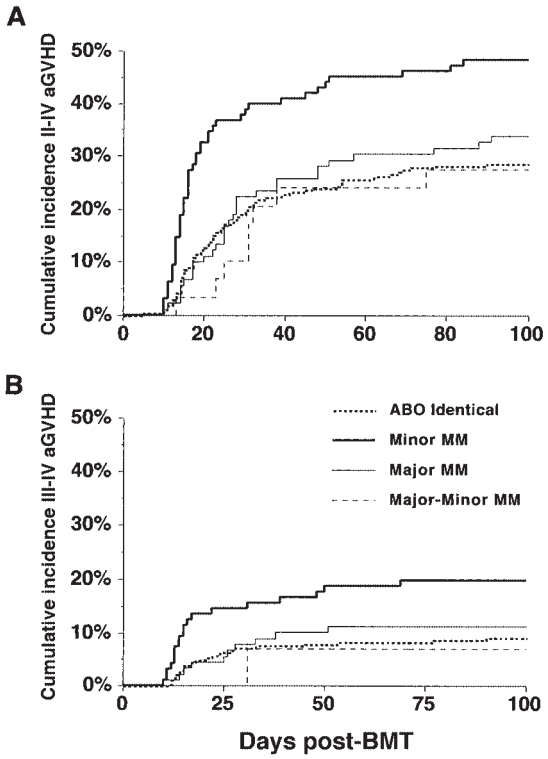

Figure 1. Cumulative incidence of aGVHD based on ABO matching. The cumulative incidence of aGVHD (grades II to IV) (A) and severe aGVHD (≥grade III) (B) is shown based on ABO matching between donor and recipient. The overall 100-day incidence of grades II to IV GVHD is 33% and of severe aGVHD is 11%.

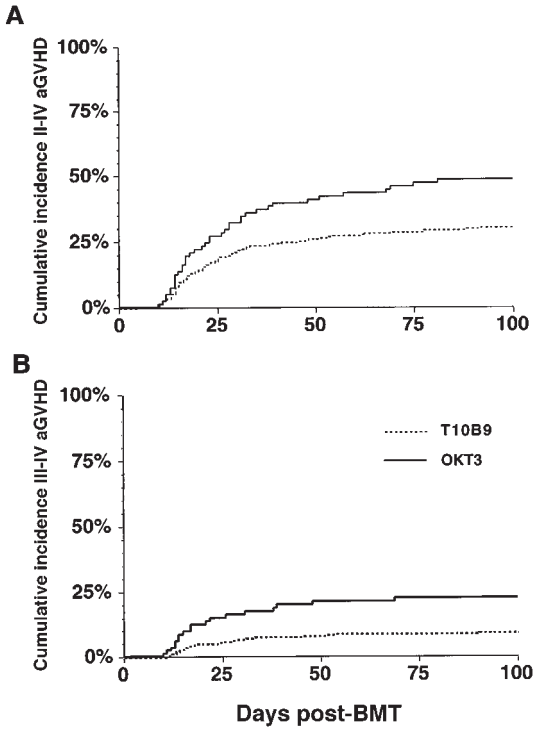

Figure 2. Effect of antibody used for TCD on aGVHD. The cumulative incidence of aGVHD (grades II to IV) (A) and severe aGVHD (≥grade III) (B) is shown, based on monoclonal antibody used for TCD.

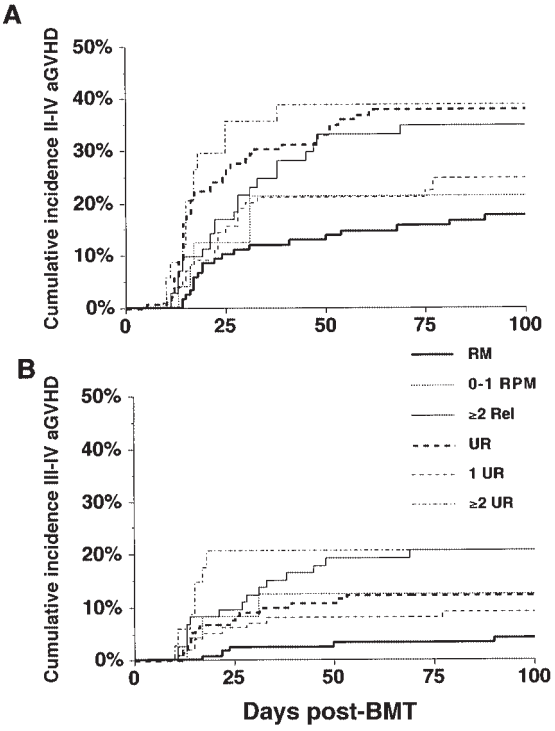

Figure 3. Effect of HLA mismatching on aGVHD. The cumulative incidence of aGVHD (grades II to IV) (A) and severe aGVHD (≥ grade III) (B) is shown, based on HLA mismatch. RM indicates related matched siblings; 0-1 RPM, 0 to 1 Ag MM related donor; ≥2 Rel, 2 or more Ag MM related donor; UR, matched unrelated donor; 1 UR, 1 Ag MM unrelated donor; ≥2 UR, 2 or more Ag MM unrelated donor.

Survival based on HLA matching and donor source was compared to the genotypically HLA-identical sibling reference group. There was no significant difference in survival for patients receiving a graft from a 0 to 1 Ag MM related donor or a fully matched UR donor. Recipients of grafts from 1 Ag MM UR donors did have a higher risk of death (RR = 1.62, $P = .01$). However, mismatching for 2 or more HLA alleles was most strongly associated with decreased survival for both recipients of related (RR = 2.12, $P < .001$) and UR grafts (RR = 2.25, $P = .001$), as shown in Table 4 and illustrated in Figure 4A. The effect of HLA match grade is also seen when the high-risk group is excluded from the analysis, as shown in Figure 4B. CMV status at the time of transplantation was associated with decreased survival only for the combination CMV-negative donor to CMV-positive recipient (RR = 1.50, $P = .02$) compared to the negative recipient, negative donor group after adjustment for other variables. Decreased survival was also associated with the disease risk group, with both intermediate- (RR = 1.48) and high-risk groups (RR = 2.02) showing a significant difference compared with the standard risk group. The survival of the 34 patients who could not be assigned to a risk group was not different from those in the standard risk group.

DISCUSSION

Several factors from our analysis were found to be associated with transplantation outcome after TCD BMT; some were expected, and others not. As expected, a mismatch for 2 or more HLA alleles was associated with increased aGVHD risk and lower survival rates, whereas older recipient age and CMV-negative donor to CMV-positive patient grafts were associated with extensive cGVHD and lower survival rates. Likewise, survival was affected by the disease status, with the highest survival rates seen for the standard risk group. Somewhat unexpected were the findings that minor ABO incompatibility and the antibody used for TCD were independent risk factors for aGVHD. Also of significance were the factors that were not associated with poor outcome in our patient group. In particular, patient age was not associated with an increased risk of aGVHD when analyzed as a discrete variable or as a continuous variable (data not shown),

Table 3. *Multivariate Analysis for Extensive Chronic GVHD*

Variable	RR (95% CI)	P
Recipient age, y		
≤20	1.0	
>20	2.23 (1.60-3.10)	<.001
Recipient-donor CMV status		
Negative-negative	1.0	.15*
Negative-positive	1.26 (0.83-1.94)	.28
Positive-negative	1.86 (1.25-2.77)	.002
Positive-positive	1.35 (0.87-2.08)	.18

*Three *df* test.

Table 4. *Multivariate Analysis for Survival*

Variable	RR (95% CI)	P
Recipient age, y		
≤20	1.0	
>20	1.63 (1.25-2.13)	.001
HLA mismatch		
Genotypically matched related	1.0	<.001*
0-1 Ag related	1.49 (0.81-2.75)	.19
≥2 Ag related	2.12 (1.43-3.15)	<.001
Unrelated matched	1.12 (0.78-1.62)	.54
1 Ag unrelated	1.62 (1.10-2.37)	.01
≥2 Ag unrelated	2.25 (1.38-3.69)	.001
Recipient-donor CMV status		
Negative-negative	1.0	.09†
Negative-positive	1.25 (0.89-1.76)	.19
Positive-negative	1.50 (1.08-2.08)	.02
Positive-positive	1.29 (0.90-1.84)	.15
Disease risk group		
Standard	1.0	<.001‡
Intermediate	1.48 (1.1-1.96)	.008
High	2.02 (1.49-2.74)	<.001
Others	0.83 (4.46-1.52)	.55

*Five *df* test.
†Three *df* test.
‡Two *df* test.

even though such an association has been reported for older patients receiving grafts that were not T cell depleted [30,31]. Our approach of partial TCD may have eliminated this age-associated increased risk of aGVHD and may have permitted disparity at a single HLA allele without more GVHD for recipients of both related and UR grafts. Although recipients of matched UR donors had more aGVHD, this finding did not translate to decreased overall survival, and it resulted in only a marginally higher risk of death for recipients of 1 Ag MM UR grafts.

The assessment of the relevance of HLA matches in graft outcomes in our study as well as in most of the current published studies is complicated both by the definition of what constitutes a match and by the resolution of the techniques used to define the HLA alleles. Our program was among the first to adopt molecular techniques to define HLA class II DRB1 and DQB alleles [32]. All of the 481 donor/recipient pairs included in this study underwent molecular HLA-DRB1 and DQB typing either by oligotyping or DNA sequencing; thus, the typing at these loci was well defined. In contrast, class I HLA-A and HLA-B locus alleles were defined only by serology in 165 (46%) of the 360 pairs who were not genotypically identical siblings. The other 195 pairs were typed for HLA-A and HLA-B either retrospectively (n = 130), using full-length DNA sequencing [29] and/or 1-dimensional isoelectric focusing, or prospectively (n = 65), by DNA sequencing of exons 2 and 3 to provide a higher resolution of typing. Because of the retrospective class I typing, we identified 34 recipients of UR donor grafts that were mismatched for 2 or more HLA alleles and could be included in the analysis as a separate group. Although the HLA alleles were more fully typed in this study than in most reported studies, the true degree of HLA allele matching is not known for one third of 481 pairs for the 4 loci considered for matching. From a preliminary assessment of patients that have been sequenced at HLA-A and HLA-B, we would expect additional disparities in approximately 25% of the pairs thought to be matched by serology, although few additional disparities were identified through DRB1 or DQ sequencing compared with oligotyping (unpublished observations). Given these caveats, we found there was no significant difference between the matched sibling baseline group and the group with phenotypically identical or 1 Ag mismatched related donors in the risk of aGVHD or the survival rate. However, the rates of aGVHD and mortality were significantly higher for recipients of both related and UR donors if ≥2 HLA alleles were mismatched. Recipients of matched UR donor grafts were at higher risk of aGVHD than the matched sibling group, as has been previously reported [3], although this did not lead to increased mortality. The increased risk of aGVHD in the matched UR group might be explained by a higher degree of mismatching for minor histocompatibility antigens, because these antigens are less likely to be matched between donor and recipient outside the family setting. Unexpectedly, recipients of 1 Ag MM UR donor grafts did not have a significantly higher risk of aGVHD in our study. Approximately 11% of the patients in this group received ATG compared to 6% in the UR matched group, a factor that might account for some of the reduction in aGVHD. It could be argued that residual host immunity might have targeted and eliminated HLA-incompatible donor–derived T cells in this setting,

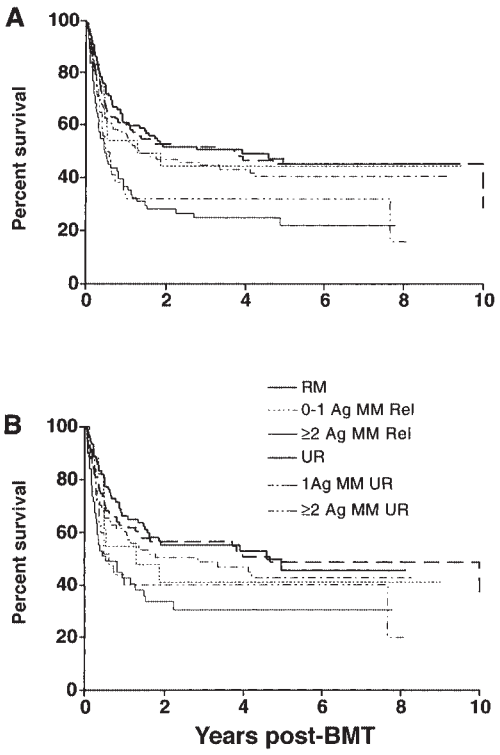

Figure 4. Probability of survival based on HLA matching. The probability of survival based on HLA mismatch is shown for the entire dataset (A) and with the exclusion of the 116 high-risk patients (B). The 3-year overall survival rate for the entire group is 44% (95% CI, 40%-49%).

thus resulting in less aGVHD in this group than in the HLA matched UR group. Unlike the matched UR group, recipients of 1 Ag MM UR grafts were at a marginally increased risk of death compared with the baseline matched sibling group (RR = 1.6, P = .02). However, the validity of these findings will require that the true class I identity of both the matched UR and the 1 Ag MM UR groups be resolved through high-resolution molecular typing. These studies, additionally including the potential role of HLA-C matching in transplantation outcome, are ongoing.

The effect of minor ABO incompatibility on the rate of aGVHD was also unexpected. No differences in either GVHD or survival rates were seen between ABO matched donor/recipient pairs, major ABO mismatched pairs, or major-minor ABO mismatch (A into B or B into A) pairs. However, recipients of minor ABO mismatched grafts had an RR of aGVHD twice that of the baseline ABO matched pairs. A similar finding was reported by Bacigalupo et al. [33] in a study of 174 recipients of unmodified marrow allografts, although earlier studies at other centers did not indicate an association of ABO and aGVHD in this setting [34,35]. In the Bacigalupo et al. study, ABO match was the only significant correlate to grades II to IV aGVHD in a Cox proportional hazard model, with recipients of minor mismatched grafts having the highest incidence of aGVHD (82%), whereas the ABO major mismatched group had the lowest incidence (39%). However, in studies published since 1988, ABO has rarely been indicated as a risk factor for aGVHD [36], but most studies appear not to have included ABO as a variable in these assessments. A recent study published in abstract form by Stussi et al. did demonstrate a higher risk of aGVHD associated with minor ABO incompatibility (RR = 3.09, P = .005) in a study of 562 recipients of allogeneic hematopoietic stem cell transplants [37], confirming our findings. ABO incompatibility as a risk factor for survival after hematopoietic stem cell transplantation has been described, with recipients of grafts with a major ABO incompatibility having the worse outcome [38-40]. Neither this finding nor decreased survival rates in pairs with a major-minor mismatch as described by Stussi was seen in our patient group. The mechanism behind the association of minor ABO disparity with aGVHD is not clear. It may be that the glycotransferase enzymes coded for by the A or B genes not present in the donor serve as minor histocompatibility antigens that can be targets for T cells mediating aGVHD. However, our own data argue against this hypothesis, because increased aGVHD risk was not seen in patients with a bidirectional mismatch. A role for donor-derived antibodies to A or B blood group antigens is unlikely, because the processing for TCD removes all donor plasma, and donor B-cell engraftment and antibody production in recipients of TCD grafts generally occurs outside the window for onset of aGVHD [8,9]. Clearly, the significance of ABO compatibility as a risk factor for aGVHD requires further study to confirm our findings and those of Stussi et al. and Bacigalipo et al. Given the strong statistical correlates found in the 3 studies linking ABO matching to aGVHD, ABO matching should be considered at the time of donor selection.

The third risk factor that was independently associated with aGVHD in our study was the MoAb used for TCD. The majority of transplantation performed at our center used $T_{10}B_9$, a nonmitogenic immunoglobulin M murine MoAb, as the purging reagent [29,41]. Our studies have indicated that $T_{10}B_9$ binds to both T-cell receptor (TCR)$\alpha\beta$ and TCR$\gamma\delta^+$ T cells at concentrations used for purging, when flow cytometry is performed using a biotinylated form of the antibody [42]. If used as a fluorescein isothiocyanate conjugate, $T_{10}B_9$ binding is restricted to TCR$\alpha\beta^+$ T cells (unpublished results; [43]), a phenomenon previously described for other CD3-specific antibodies [44]. However, when $T_{10}B_9$ is used to deplete T cells by complement-mediated lysis, the TCR$\gamma\delta^+$ T-cell subset is selectively spared (0.5 log depletion versus 1.9 log depletion of the TCR$\alpha\beta^+$ T-cell subset) [19,42]. This selective sparing of TCR$\gamma\delta^+$ T cells was not seen for marrow TCD with OKT3 in our preclinical studies [42] or in assessment of the grafts received by the patients in this study (median 1.1 log depletion of TCR$\gamma\delta^+$ T cells). Despite the greater depletion of TCR$\gamma\delta^+$ T cells by OKT3 in our hands, the overall degree of TCD using OKT3 was found to be less than that attained with $T_{10}B_9$, resulting in a larger median dose of T cells for recipients of grafts that were T cell depleted with OKT3. In earlier studies restricted to recipients of grafts that were T cell depleted using $T_{10}B_9$, we identified T-cell dose as a risk factor for aGVHD in recipients of related allografts but not in recipients of unrelated allografts [19]. In contrast, T-cell dose as a single factor was not independently correlated with aGVHD in this study. The reason for the higher risk of aGVHD in recipients of grafts that were T cell depleted with OKT3 may reflect not only the higher dose of T cells infused and a difference in T-cell subset content, but also fact that OKT3 is mitogenic and may actually stimulate and promote survival of the residual T cells. In contrast, the residual T cells in grafts that were T cell depleted with $T_{10}B_9$ may be more likely to undergo apoptosis and not survive to cause aGVHD [41]. Despite the higher rate of aGVHD, the MoAb used for TCD was not an independent risk factor for survival.

The nature of cGVHD in the setting of partial TCD may differ from conventional non-TCD transplantation. Our own observation is that most patients, adult and pediatric, with limited cGVHD are very responsive to therapy and experience little morbidity as a result, as we have reported for our pediatric patients [18]. For this reason, we chose extensive cGVHD as our endpoint for assessing cGVHD. Both older patient age (>20 years) and a positive patient/negative donor CMV status at transplantation were significant risk factors for cGVHD and for lower survival rates. Older patient age has been widely described in a variety of transplantation settings as a risk factor for cGVHD [45,46]. The reason is likely multifactorial and may involve the impaired immune status of older patients as well as the higher likelihood of preceding infectious complications, particularly with the herpes virus family [47,48]. Thymic function, in particular, is required for the generation of new immune responses and for the development of specific T cells that regulate the patient-directed alloreactivity of engrafted donor T cells [49]. Recovery of thymic function, as represented by the appearance of naive CD4$^+$ T-cell subsets [50] and T-cell receptor excision circle (TREC)$^+$ T cells [51], is more impaired in older recipients of hematopoietic stem cell transplants. TREC$^+$ T-cell levels are especially low in

patients with cGVHD [52,53]. Moreover, overall recovery of immune function, both humoral and cellular, is superior in younger patients. Younger age, together with better restoration of thymic function, may contribute to the increased survival rates and lower likelihood of extensive cGVHD seen in our study for patients aged ≤20 years [54-57].

Patients with a positive CMV status at the time of transplantation have been previously described as having an increased risk for aGVHD and a lower rate of survival following both conventional [58-61] and partially TCD transplantation [62,63]. The association of a positive patient CMV status with cGVHD has been less often described [47]. We did not find an association between CMV status and aGVHD in our TCD patient group. By univariate analysis, we saw a higher rate of extensive cGVHD and lower survival rates for CMV-positive recipients with either CMV-positive or CMV-negative donors compared with CMV-negative patients (not shown). However, after adjustments for other significant variables, only the seropositive recipients of seronegative marrow remained at a higher risk for extensive cGVHD and lower survival rates. The association of CMV and cGVHD may be explained by the presence of latent human CMV in many of the target organs for cGVHD, including the liver, the spleen, and the endothelium [64]. CMV infection may result in the up-regulation of adhesion molecules secondary to inflammatory cytokine production during periods of viral reactivation posttransplantation [65,66]. The inflammatory cytokines produced during CMV infection may help to initiate or intensify tissue injury mediated by alloreactive donor-derived T cells, thus leading to higher rates and/or degrees of cGVHD. Indeed, preceding CMV infections have been found to be associated with onset or exacerbation of cGVHD[48]. The higher mortality of CMV-positive patients receiving transplants from CMV-negative donors in our patient group may reflect the need for prolonged immune suppression because of cGVHD as well as the posttransplantation delay in recovery of cellular and humoral immunity to CMV described for such patients [67,68]. This characteristic would leave these patients at higher risk for developing CMV disease and its resultant morbidity and mortality [62,69].

Differences in significant risk factors for GVHD and survival that were found in our patient group compared with patients in other studies likely reflect the use of partial TCD as well as other aspects of our conditioning and posttransplantation regimens. We have demonstrated that the outcome of TCD transplantation can be influenced by the antibody used for TCD, even within the narrow specificity group defined in the recent IBMTR study [4]. We and others who perform transplantation using a similar approach can use the findings of this study not only to better inform patients, but also to identify treatment options that are more likely to achieve the best outcomes. Our findings should lead to improved algorithms for donor selection that consider as favorable the choice of a CMV-positive donor for CMV-positive patients and that avoid a minor ABO mismatch. Improved HLA typing methods should allow us to identify with more confidence donors with no more than single HLA disparities and better avoid those with 2 or more disparities, a donor characteristic that clearly compromises outcomes. It is only through this process of identifying risk factors for a particular patient group that improvement in the quality of transplantation can be achieved.

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